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Laser-activated Solid Protein Bands for Peripheral Nerve Repair: An In Vivo Study

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Background and Objective: **Severed tibial nerves in rats were repaired using a novel technique, utilizing a semiconductor diode-laser-activated protein solder applied longitudinally across the join. Welding was produced by selective laser denaturation of solid solder bands containing the dye indocyanine green.**
Study Design/Materials and Methods: **An in vivo study, using 48 adult male Wistar rats, compared conventional microsuture-repaired tibial nerves with laser solder-repaired nerves. Nerve repairs were characterised immediately after surgery and after 3 months.**
Results: **Successful regeneration with average compound muscle action potentials of 2.5 ± 0.5 mV and 2.7 ± 0.3 mV (mean and standard deviation) was demonstrated for the laser-soldered nerves and the sutured nerves, respectively. Histopathology confirmed comparable regeneration of axons in laser- and suture-operated nerves.**
Conclusion: **The laser-based nerve repair technique was easier and faster than microsuture repair, minimising manipulation damage to the nerve. Lasers Surg. Med. 21:134–141, 1997.**

Key words: indocyanine green dye; laser-activated protein solder; nerve repair; tissue welding

INTRODUCTION

Lasers are increasingly used for tissue welding [1–15] in experimental surgical procedures. In direct laser anastomosis of tissues, proteins within the target tissue are coagulated to form a bond joining the two edges [1,2]. This technique offers advantages over conventional microsuturing in that it involves less suture or needle trauma and decreased foreign body reactions [3]. It is also simpler and faster to perform in most instances. An example is the laser welding of peripheral nerves, which offers the potential for faster operations and improvements in the healing of nerve grafts or other nerve repairs when compared with suturing, which inevitably results in some tissue fibrosis surrounding the repair site. However, in some cases, direct laser welding is unsatisfactory [4] and has a high failure rate. Some workers have used temporary or permanent stay sutures [5,6] to improve the success rate of laser anastomoses as the welds tend to be weakest in the first few days postoperatively [3]. This does not eliminate the sutures and their consequent problems and also complicates the operative procedure.

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TABLE 1. Breakdown of 48 Experimental Rats Used in Different Components of This Study

	Total number of rats	Light microscopy immediately after surgery	Tensile strength immediately after surgery	Light microscopy and electrophysiology 3 months after surgery
Laser repair	24	5	5	14
Suture repair	24	5	5	14

An alternative solution is to add extra protein in the form of a "solder" to the tissues to supplement the bond. Protein solutions such as albumin or fibrinogen have been used successfully in conjunction with a CO_2 laser to heat the fluid solder [7]. Another approach is to apply an extra layer of tissue, such as epineurium in nerve repair (8), to the anastomosis site. This approach also avoids foreign body reactions, but suitable tissue must be prepared. Some workers also have used solder to help seal welded tissue against fluid leakage [9].

To date, infrared lasers, such as Tm, Ho: YAG, and CO_2 at wavelengths of 2.0 μm and 10.6 μm, respectively, have been primarily used for direct laser tissue welding [4, 10, 11] and for welding using applied protein solder [7–9]. The choice of such lasers is based on absorption of the laser energy by water [12], thereby heating proteins within the target tissue or the applied solder, resulting in coagulation at ~70°C. The resulting denaturation of protein is not specific to the target tissue or solder, and all irradiated tissues are heated. This is a problem when repairing peripheral nerves as axons can be damaged when the epineurium is welded. Some workers have used dyes at the weld site [13] to avoid most of the "collateral" thermal damage. The dye is applied directly to the tissue, or mixed with the applied protein solder [14–16]. The laser wavelength is chosen to be strongly absorbed by the dye, but poorly absorbed by water and bodily tissues. In our study, this approach has been used. An 800 nm diode laser is used to denature the dye-protein solder applied to the perineurium, with energy transferral due to absorption in the dye, indocyanine green [14–17], contained within the albumin solder. Thus there is negligible thermal damage to the adjacent nerve tissue. The diode light is delivered by optical fiber in a handheld fiber chuck, so it easily may be incorporated into the microsurgical procedure. To simplify the operation further, we have developed an easy to handle solid protein solder in the form of bands.

Although laser anastomoses of blood vessels and other tubular tissues may be tested for patency immediately, satisfactory nerve repair depends on axonal regeneration [18], which may take many weeks. In this report, we compare in vitro weld strength measurements and in vivo electrophysiological measurements and histopathology observations 3 months after surgery for laser-cured protein solder nerve repairs and conventional microsutured nerve repairs.

MATERIALS AND METHODS

A total of 48 young adult male Wistar rats (400–550 g) were used in this study. Twenty-four rats received laser solder nerve repair and the remainder received standard suture repair, as detailed below. Five laser and five suture-operated rats were used for tensile strength measurements, and a further ten rats were used for light microscopy studies. The remaining 28 rats were subjected to a study of functional recovery using serial electrophysiology and histopathological examination after three months. These numbers are summarised in Table 1.

A GaA1As laser diode with a nominal power of 250 mW and wavelength of 800 nm (Spectra Diode Labs, San Jose CA) was used to denature the protein solder. The laser radiation was coupled into a 100-μm diameter core optical fiber, which was held by hand in a fiber chuck. The diode current and temperature were controlled by a SDL-800 diode driver. The diode was operated in continuous mode at 90 mW during the laser soldering, with a spot size at the tissue of 200 μm diameter, corresponding to a maximum irradiance of 300 W cm^{-2} at the tissue. The laser power was measured with a Scientech power meter (Boulder, CO). Because this laser is Class IIIb, and not eye safe, protective glasses were worn at all times during the procedure.

The solder used in this study consists of concentrated bovine serum albumin and the dye indocyanine green (ICG) (Becton Dickinson, MO) (0.2% by weight), mixed in water. (Indocyanine green has a maximum absorption coefficient at a

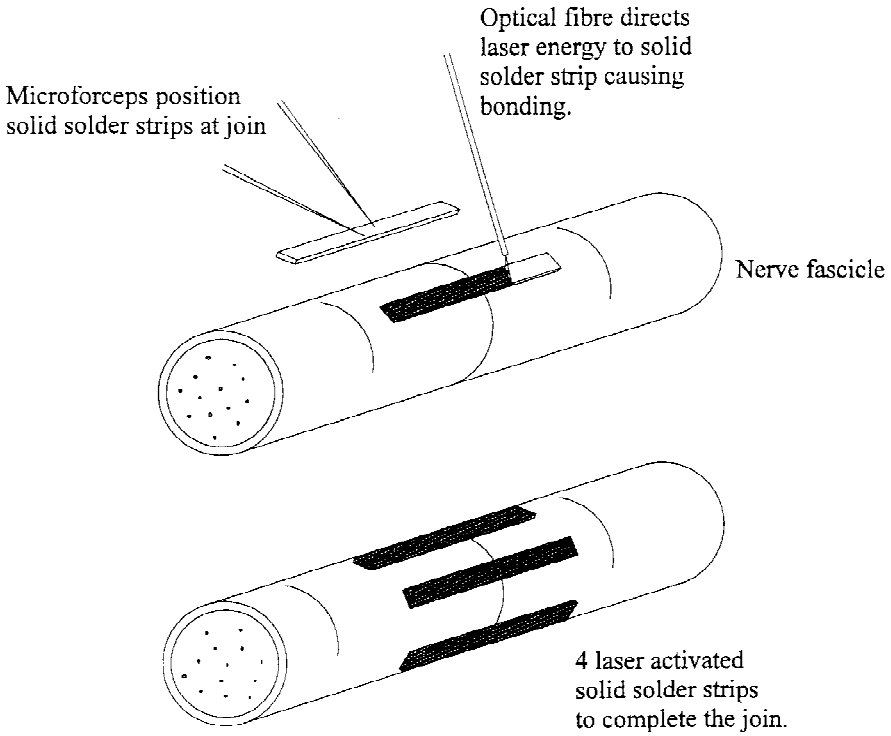

Fig. 1. Diode laser light delivered by optical fibre to heat the protein solder bands applied to the repaired nerve.

wavelength of 805 nm of 2×10^5 M^{-1} cm^{-1}.) This dye binds preferentially with proteins [17] ensuring that heat is efficiently transferred to denature the solder. The solder was formed by mechanical pressure into thin slabs, and cut into thin solid bands (dimensions 3 mm × 0.5 mm × 0.15 mm) soon after mixing, and these were allowed to dry. They were stored until use between inert metal plates in a cool, dark, dry place. The bands were composed of sterile materials, which were handled and mixed using sterile instruments. No further sterilisation was carried out on the bands.

Prior to surgery, anaesthesia was induced and subsequently maintained using a mixture containing halothane (4% during induction, 2% thereafter) in O_2 (1 L/min). An OPMI 7 operating microscope (Zeiss, Germany) was used throughout the surgery. The sciatic nerve of the left leg was exposed distal to the sciatic notch where it gives rise to the tibial, peroneal, and sural nerves. The tibial nerve was separated from surrounding subcutaneous tissues for a length of 1 cm. For a laser solder repair, a section of thin gauze material was placed under the tibial nerve to facilitate its rotation, and for the suture repair, a section of plastic was placed under the nerve to facilitate suturing. The tibial nerve was then severed using serrated micro-scissors and left for 3 minutes to permit the normal extrusion of axoplasm to occur. This was then trimmed with the micro-scissors as required, after which the nerve was repaired with either four laser solder bands, or four 10-0 nylon perineurial sutures. In both cases the epineurium was resected before repair. The laser solder method involved aligning both stumps of the severed nerve with micro-forceps, apposition of a 3 mm long band of solder longitudinally across the junction of the severed ends, and finally creation of a strip weld by denaturing the solder with the diode laser output from the optical fibre used in a continuous pass of duration 40 ± 10 s. This is portrayed schematically in Figure 1. At a diode output power of 90 mW, the solder was observed to turn brown on its surface and opaque underneath from the single pass, signalling denaturation. The gauze under the nerve was then manipulated to rotate the nerve so that three similar bands could be applied, each ~90° apart. For all operations, the time of repair was recorded and a photograph was taken for later reference. The skin wound was apposed with three sutures of 3-0

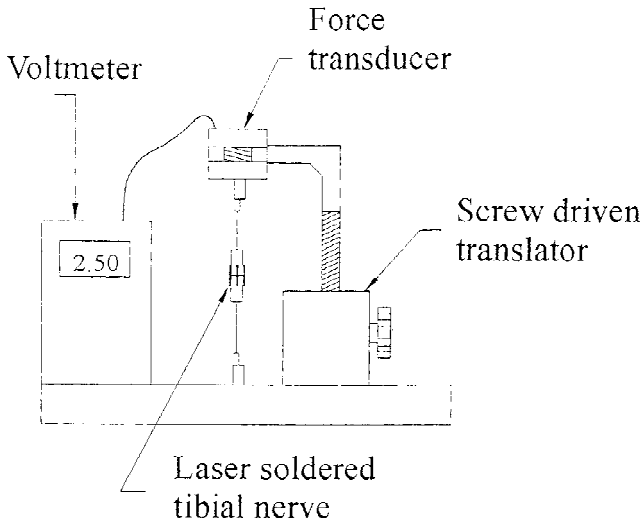

Fig. 2. Experimental arrangement for tensile strength measurements of repaired nerves.

polypropylene. Rats were placed in their cages with no restriction of movement for 3 months.

Ten of the operated rats had a 1 cm long section of the laser- or suture- repaired nerve harvested immediately after surgery for tensile strength measurements. (Each nerve was repaired either by four solder bands or four sutures.) Fine silk was tied to each end of the tibial nerve. One end was then attached to a calibrated force transducer (FT30C, Grass Instruments, Quincy, MA) and the other to a screw driven translator, shown in Figure 2. The translator stretched the nerve slowly and progressively while the applied tension was observed on an oscilloscope connected to the force transducer. Tension was applied until the repair failed, and the breaking force was recorded. The nerves were kept moist during this procedure, as upon drying, the tensile strength can be increased [7]. For conventional light microscopy, the nerve repair site was fixed in 5% formalin, alcohol dehydrated, embedded in paraffin, sectioned (10 μm) longitudinally, and stained with either Masson's trichrome or Giemsa.

Three months postoperatively, rats were anaesthetised (as above), the surgical site exposed, and the tibial nerve inspected. The peroneal and sural nerves were then severed so that only the tibial nerve branch of the sciatic nerve could conduct electrical impulses to the distal muscles of the hind foot following proximal stimulation of the sciatic nerve [19]. The wound was then closed as before. It should be noted that the peroneal and sural nerves were not severed at the time of the initial operation, so as to allow some movement and use of the operated leg during the time of recovery of the tibial nerve.

Two days later, the rats were positioned on their side and insulated from the table by a folded surgical drape. An infrared lamp was used to maintain their rectal temperature above 36°. A clinical electromyograph (Cadwell Sierra EMG/EP) was used for stimulation and recording. Two 25-gauge stimulating electrodes were positioned 10 mm apart on each side of the sciatic nerve above the sciatic notch, near the hip. The nerve was stimulated supramaximally using rectangular pulses (0.1 to 0.3 ms duration; 0 to 30 mA amplitude; repetition rate 1 Hz). Compound muscle action potentials (CMAPs) were recorded from the plantar muscles of the foot using a set of three recording electrodes: a 30-gauge reference electrode inserted into the heel pad, a 30-gauge recording electrode inserted into the plantar muscles of the foot, and a 25-gauge ground electrode inserted subcutaneously between the stimulating and recording electrodes [20, 21]. The CMAPs were recorded on the EMG. Histopathology of the sutured and laser soldered nerves at 3 months recovery was conducted immediately following the electrophysiology studies.

Throughout the text, values given are the mean ± standard deviations. Comparisons of the duration of surgery and breaking strength of the nerve repair are made using students t-tests (unpaired). Comparisons between the amplitude, duration and latency of evolved CMAPs in sutured and laser-soldered repairs are made using a one-way analysis of variance. P values < 0.05 were considered significant.

RESULTS

At the completion of surgery, all nerve repairs were technically successful. The laser-solder operating technique was found to be easier than conventional microsuturing. This resulted in significantly shorter operating times ($P < 0.05$; t-test) for laser solder repairs (10 ± 5 min) than for microsuture repairs (23 ± 9 min). The tensile strength of five laser-solder-repaired nerves immediately after the operation (21 ± 5 g) was significantly less than the tensile strength of the microsutured nerves (50 ± 10 g).

Histopathological examination of the repair site immediately after surgery demonstrated good

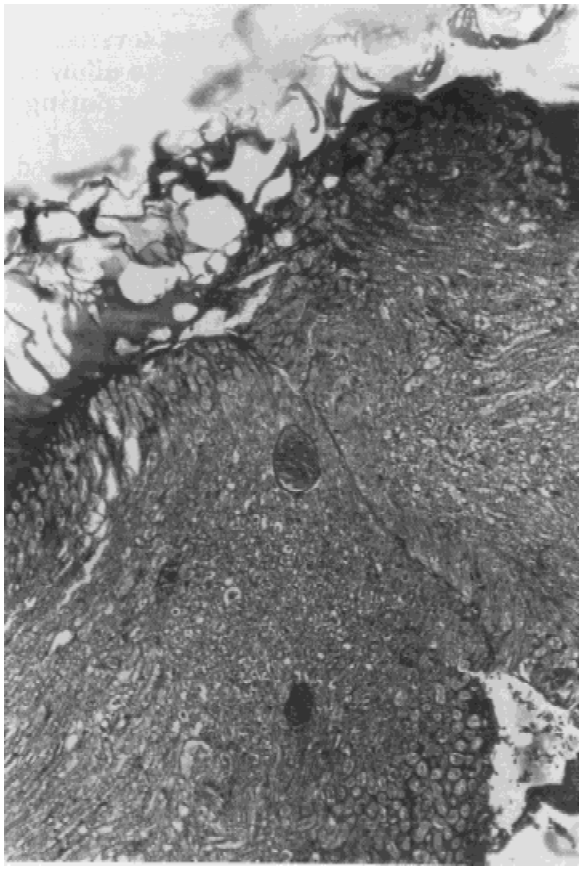

Fig. 3. Light micrograph of a longitudinal section of tibial nerve immediately after laser protein band nerve repair. The axons show no evidence of thermal damage, retaining their normal wavy appearance (Masson's trichrome, 100×).

bonding of the solder with the perineurium. The inner axons remain structurally normal, with little or no evidence of thermal injury. A tibial nerve weld produced by the diode laser and protein/dye solder is shown in longitudinal section in Figure 3. The homogeneous vacuolated material is the protein solder. Both the protein solder and the perineurium have denatured, forming the bond. On the lower side of the bond, the axoplasm maintains its normal wavy structure. Since heating is concentrated at the dye, denaturation is limited to the solder and adjacent perineurium. The axons on the edges of the fascicle are twisted and appear larger as they are in transverse section.

Electrophysiological measurements were performed on 28 rats 3 months following surgery. In this group, all laser-solder repairs and all suture repairs were intact. The average amplitude of the CMAP following supramaximal stimulation of the nerve above the repair site was 2.5 ± 0.5 mV for the laser-soldered tibial nerves and 2.7 ± 0.3 mV for the microsutured nerves. Typical evoked CMAPs are illustrated in Figures 4 and 5, and the CMAP duration, amplitude, and latency are presented in Table 2. When compared by ANOVA, the values of amplitude, duration and latency of the laser-soldered nerves and the sutured nerves were not significantly different at the 0.05 level. The CMAP evoked by supramaximal stimulation of the tibial nerve in the contralateral (right) limb was 9.1 ± 2.9 mV. This was significantly higher than the CMAPs evoked following either type of nerve repair.

Histopathology at 3 months showed regeneration of myelinated axons in laser nerve repairs with no apparent discontinuity of the nerve or the perineurium. The axon alignment was typically wavy, and there was no noticeable fibrosis at the repair site. After 3 months, it was difficult to identify the exact repair site histologically, as the protein bands had disappeared. There was no evidence of chronic or acute inflammation or myelin phagocytosis. Figures 6 and 7 show transverse and longitudinal sections of a laser-welded nerve 3 months after surgery. The sutured nerves also showed successful repair with myelinated axon regeneration, and normal axonal wavy structure, except near the nylon suture, which is surrounded by fibrous tissue. No inflammation was evident. The degree of myelination was similar in the sutured and laser-welded nerves, but less than in normal healthy nerves. There was unequivocally more fibrosis in the sutured nerves than in the laser-repaired nerves, and neuromata that were very rarely observed in the laser-welded nerves were seen to varying degrees in the sutured nerves.

DISCUSSION

The repair of a severed peripheral nerve in a human patient may involve the individual joining of forty fascicles, with three or four microsutures per fascicle. Since meticulous care is required, such an operation tends to be prolonged. We have sought an alternative method of nerve repair that is significantly faster than the present microsuture technique, but with the potential for a similar or better functional recovery. We chose to use a solid protein solder prefabricated in the form of bands. This has a number of advantages: the solder bands may be prepared in advance and have a shelf life (determined by the dye) of several weeks if stored appropriately. For clinical use, the pro-

TABLE 2. Latency, Duration, and Amplitude (mean and standard deviation) of CMAP Measurements for Control, Laser-Soldered, and Sutured Tibial Nerves

	CMAP amplitude (mV)	CMAP duration (ms)	CMAP latency (ms)
Normal nerve (contralateral control)	9.1 ± 2.9	1.25 ± 0.02	2.4 ± 0.2
Laser-soldered nerve	2.5 ± 0.5	1.86 ± 0.08	4.0 ± 0.5
Sutured nerve	2.7 ± 0.3	1.95 ± 0.12	3.8 ± 0.3

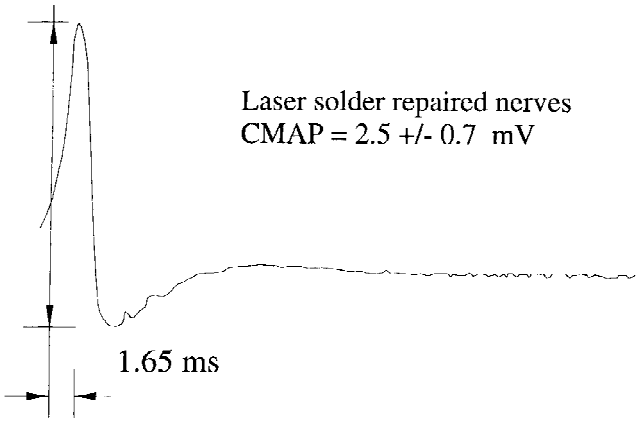

Fig. 4. Compound muscle action potential of laser-solder repaired nerves 3 months after surgery.

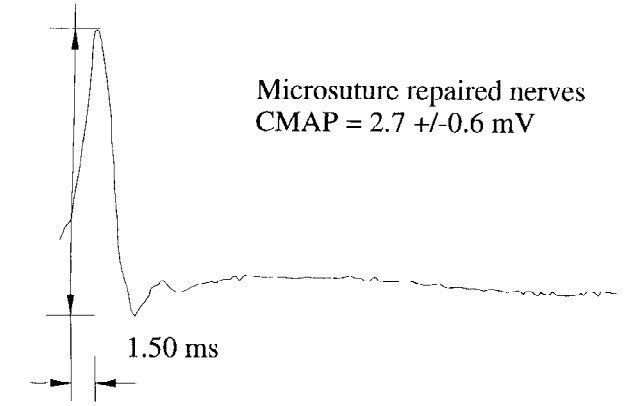

Fig. 5. Compound muscle action potential of microsuture repaired nerves 3 months after surgery.

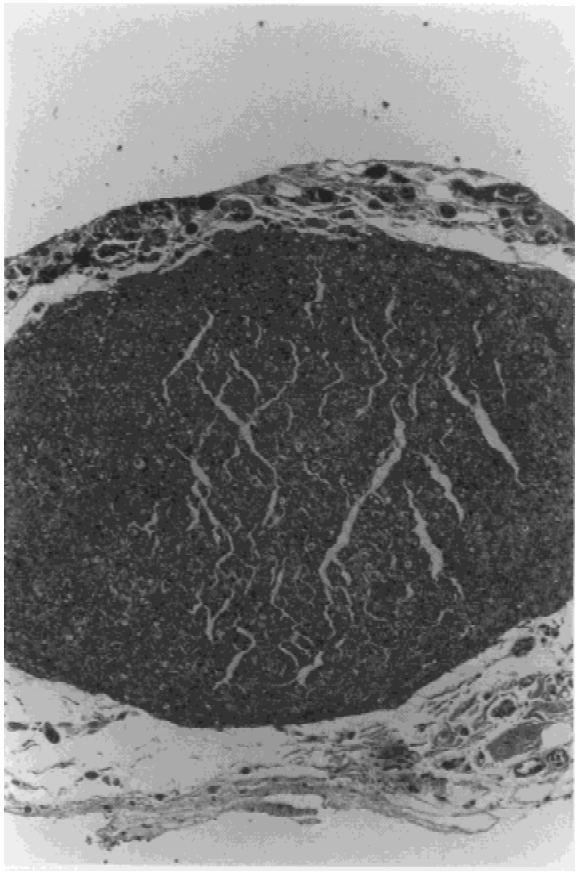

Fig. 6. Light micrograph of a transverse section of laser-solder repaired nerve 3 months after surgery (Masson's trichrome, 100×).

tein bands could be sterilised by gamma radiation after packaging. The bright green bands are easier to handle and apply to the nerve than fluid solder, and are readily manipulated with forceps. Also, since they are applied to the perineurium of the nerve, the solder bands do not obstruct regenerating axons.

A significant advantage of the laser solder technique is the absence of damage to axons beneath the denatured perineurial layer seen immediately after surgery. Three months postoperatively, comparable regeneration of laser solder and microsuture repaired nerves was evident by electrophysiological and histopathological studies. Further, evidence of foreign body reaction to suture is present in the microsutured nerves, but not in the laser soldered nerves.

A popular experimental laser-based technique of peripheral nerve repair requires overlap of the severed edges of the nerves (epineurial or perineurial membranes), and fusion of the epineurial membranes[3]. The organic solder used for strip welding does not require epineurial or perineurial overlap because of the high rigidity of the laser-denatured solder: the severed ends are

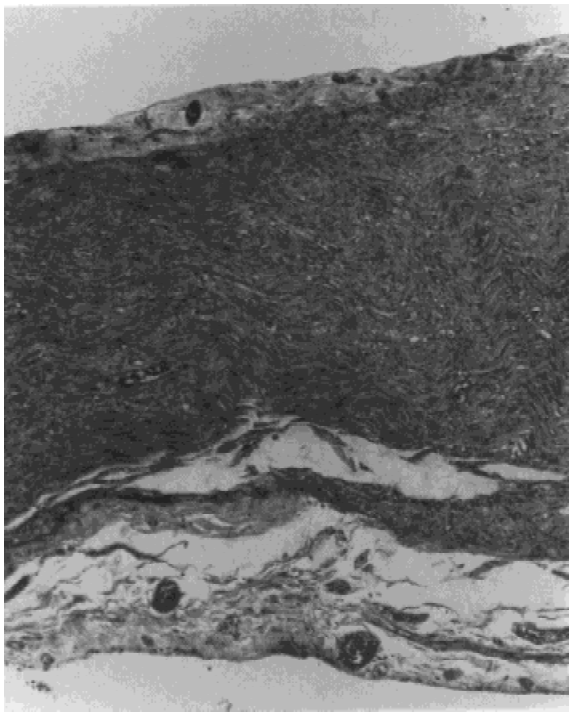

Fig. 7. Light micrograph of a longitudinal section of laser solder repaired nerve at 3 months after surgery. Nerve continuity of the axons, sheath, and perineurium has been restored (Masson's trichrome, 100×).

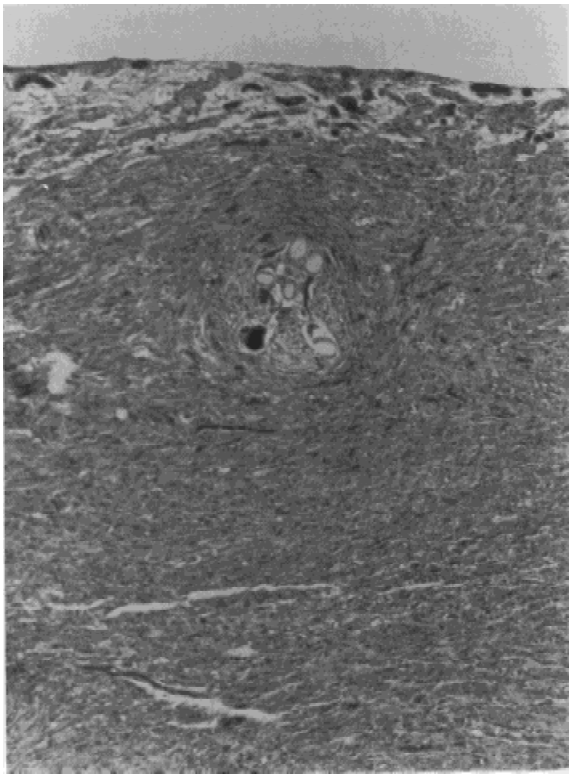

Fig. 8. Light micrograph of a longitudinal section of microsuture repaired nerve at 3 months after surgery. Some of the regenerated axons are obstructed by fibrous tissue around the suture (Masson's trichrome, 100×).

simply abutted together. This reduces manipulation of the nerve ends and so minimises mechanical damage to the axons. Thermal axonal damage is also reduced compared to THC:YAG or CO_2 laser welding as the incident laser energy is only absorbed by the indocyanine green dye contained within the solder, and not directly by the underlying tissues.

The laser-activated solid protein solder has been demonstrated as a successful method of repair for nerves less than 1 mm in diameter. Although the tensile strength achieved is only half that of the microsutured nerves, it is sufficient to allow satisfactory regeneration of the severed nerves. By contrast, in most cases of direct laser welding of the epineurial or perineurial membranes, the nerve dehiscence rate is unsatisfactory [4], unless stay sutures are used, and the organic solders used previously displayed weaker tensile strengths [7]. The application of strips of organic solder across a cut nerve, which we call the "protein band" technique, is different to the circumferentially applied solder techniques [22]. The protein band technique reduces the laser-irradiated nerve surface, as the laser energy is not applied to a broad area, but moved steadily along the strip [16].

CONCLUSIONS

Nerve anastomosis using laser-heated protein bands has promise as a simpler, faster technique than microsuturing. The use of the protein dye mixture in solid bands applied along the nerve has the advantage that the underlying nerve axons are protected from thermal damage while being held together firmly enough to allow successful regeneration. There is also potential to apply the laser cured protein bands to the repair of other tubular tissues such as in the anastomosis of blood vessels.

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